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Does Iron or Heme Control Rat Hepatic Delta-Aminolevulinic Acid Synthetase Activity

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DOES IRON OR HEME CONTROL RAT HEPATIC δ-AMINOLEVULINIC ACID SYNTHETASE ACTIVITY?

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SUMMARY

Disodium ethylenediamine tetraacetic acid and/or allylisopropylacetamide administration to rat pups did not evoke a premature induction of hepatic δ-aminolevulinic acid synthetase. Administration of iron to adult rats did not alter δ-aminolevulinic acid synthetase activity and had little inductive effect on heme oxygenase activity. Both heme and cobalt/dextran rapidly induced microsomal heme oxygenase by 3-8 fold. Induction of heme oxygenase by heme could be totally blocked by concurrent administration of cycloheximide. These results argue against the hypothesis that iron is the physiological mediator of δ-aminolevulinic acid synthetase activity.

INTRODUCTION

Four different models for the control of hepatic heme levels have been proposed. In the classical model of Granick (1) heme represses ALA synthetase (Model I of Figure 1). Iron has been postulated to be the physiological mediator of heme levels by either inducing heme oxygenase (Model II) or by directly repressing ALA synthetase (Model III) (2,3). Recent dose response studies from this laboratory have suggested that bilirubin might be the physiological regulator of ALA synthetase (Model IV) as i) bilirubin decreases mitochondrial ALA synthetase activity at low doses (4) and ii) bilirubin may be present at a higher concentration in hepatocytes than heme.

In rat liver the activity of ALA synthetase is high before birth and falls to adult levels by day 3 postpartum (5). Unlike the adult

Abbreviations used: EDTA, ethylenediamine tetraacetic acid disodium salt; ALA, δ-aminolevulinic acid; AIA, allylisopropylacetamide.

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enzyme fetal rat hepatic ALA synthetase is not inducible by allylisopropylacetamide (AIA) or repressible by heme (6). In the neonate, rat hepatic ALA synthetase is not inducible by AIA administration alone (3).

The present studies examined the possibility of premature ALA synthetase induction in the neonatal rat pup following administration of the metal chelator EDTA and AIA. One report of such an effect has been interpreted as evidence that iron is the physiological regulator of ALA synthetase (3). Direct administration of iron via subcutaneous or intraperitoneal injection to adult male rats was used to determine the effects on total liver iron levels, ALA synthetase and heme oxygenase activities. As iron was a weak inducer of heme oxygenase, both heme and cobalt/dextran treatments were used to confirm the inducibility of this catabolic enzyme. The results of these studies are discussed in regards to the four proposed models for the control of hepatic heme levels.

METHODS

Neonatal (day 3 postpartum) or adult male Sprague-Dawley rats (170-250 g, Charles River Laboratories) were used. Adult rats (CD strain) were starved 24 hours before the administration of any compound and animals were deprived of food until sacrifice. In experiments involving three day old rat pups the offspring were separated from their dams in the afternoon of day 2 and maintained at room temperature until eventual sacrifice on the morning of day 3.

Allylisopropylacetamide was a gift from Hoffman La Roche, Inc. Hemin chloride was obtained from Calbiochem and ferric chloride and cobaltous chloride from K&K Laboratories. All other chemicals were of reagent grade and obtained from standard commercial sources. Ferric chloride or cobaltous chloride/dextran solution (a 5:1 weight ratio of dextran per metal chloride) were prepared fresh just before injection. Hemin chloride was dissolved in 0.1 N NaOH and titrated to pH 7.5 before intravenous injection. Bilirubin (Porphyrin Products) was complexed with 0.5% rat albumin in 0.9% saline and injected intravenously. ALA synthetase and HO activities were not altered by administration of any of the vehicles used.

Assay of enzyme activities. Subcellular fractions were prepared by the method of Kitchin and Woods (7). ALA synthetase activity was determined by the method of Scholnick *et al.* (8) as described by Woods (6). Post-mitochondrial supernatant or microsomal heme oxygenase activities were determined in 0.1 M potassium phosphate buffer, pH 7.4 with 0.4 mM NADPH using 20 μM heme as the substrate. Protein concentrations were determined by the method of Lowry *et al.* (9).

TABLE I

Effect of EDTA and AIA on Neonatal Rat Hepatic Mitochondrial ALA Synthetase

Treatment			ALA synthetase
Day 1	Day 2	Day 2	nmole/mg protein/hr
Vehicle	Vehicle	Vehicle	2.12 ± 0.20 (9)
EDTA	EDTA	Vehicle	2.52 ± 0.38 (9)
EDTA	EDTA	AIA	3.34 ± 0.63 (9)
EDTA (to dams)	EDTA (to dams)	AIA	3.69 ± 0.99 (6)
-	-	AIA	2.74 ± 0.45 (9)

Groups of newborn rat pups (5-7 g) were orally fed 0.1 ml of 100 mM EDTA (pH 7.5) or vehicle on day 1 and the morning of day 2 as indicated in Table 1. In one group the dams were injected with 50 mg/kg of EDTA (pH 7.5) intraperitoneally. Sixteen hours before sacrifice (afternoon of day 2) rat pups were injected with 300 mg/kg of allyl-isopropylacetamide or vehicle subcutaneously.

Values are means ± SEM for 6-9 independent samples. No comparisons were statistically significant.

Data are presented as the mean ± SEM for N observations. Data were analyzed for statistical significance by the Students t-test (10).

RESULTS

As shown in Table 1 no statistically significant differences were observed in neonatal mitochondrial ALA synthetase activities in the ALA synthetase premature inducibility experiment. These indirect studies did not provide evidence suggesting that iron was a physiological regulator of hepatic ALA synthetase activity (Model III of Figure 1). Thus iron injection via the i.p. and s.c. routes to adult male rats were used in an effort to demonstrate hepatic induction of heme oxygenase activity or repression of ALA synthetase (Models II and III of Figure 1). No significant differences were found in total liver Fe levels or ALA synthetase activity regardless of the dose, time of administration, or route (Table II). Iron/dextran (324 μmol/kg, ip) significantly increased post mitochondrial supernatant heme oxygenase activity at 4 ($P<.05$) and 24 ($P<.01$) hours after administration.

As large doses of iron did produce some small heme oxygenase induction further studies were done with equivalent molar doses of

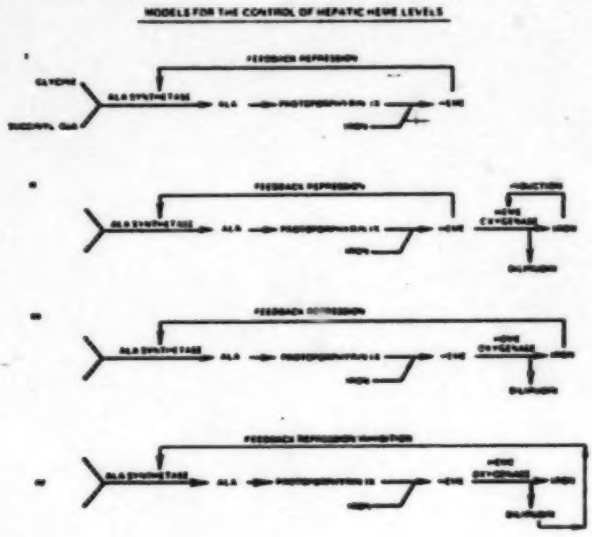

FIGURE 1

cobalt chloride/dextran and 1/10 the molar dose of heme to determine their effects on microsomal heme oxygenase activity. Table III shows that Co/dextran and heme both significantly induce heme oxygenase at 4 hours. Heme has a greater effect than Co/dextran at 4 hours while Co/dextran produced a higher heme oxygenase induction at 24 hours. Similar results were obtained with post mitochondrial supernatant preparations. Bilirubin administration (32.4 μmoles/kg, iv) to adult rats 4 hours before sacrifice caused a decrease in mitochondrial ALA synthetase activity from 0.86 ± 0.15 (controls) to 0.36 ± 0.20 nmoles ALA formed/mg protein/hr.

DISCUSSION

In previous dose response studies performed in this laboratory both bilirubin and heme significantly reduced adult rat hepatic ALA synthetase activity (4). At intravenous doses of 32.4, 3.2, and 0.32 μmoles/kg of heme, rat hepatic ALA synthetase activity was 43, 50 and 72% of control values; at the same three doses bilirubin administra-

TABLE II

Effect of Iron/Dextran Administration on Hepatic Iron Levels, ALA Synthetase and HO Activities in Adult Rats

Iron-dextran treatment					
Time (hr)	Route	Dose (μmole/kg)	Liver Iron Level (μg/g)	Mitochondrial ALA synthetase (nmole/mg protein/hr)	Post Mitochondrial Supernatant Heme Oxygenase (nmole/mg protein/10 min)
4	sc	Vehicle	79.5±13.7 (10)	0.62±.13 (8)	0.16±.02 (9)
2	sc	32	78.9±10.9 (6)	1.05±.17 (5)	0.17±.02 (5)
4	sc	32	98.4±12.5 (6)	0.88±.31 (5)	0.14±.03 (5)
4	ip	32	61.3±11.2 (6)	0.90±.21 (5)	0.20±.03 (5)
4	sc	324	68.3±18.3 (6)	0.61±.16 (5)	0.17±.02 (5)
4	ip	324	82.5±18.4 (6)	0.69±.06 (5)	0.23±.02[a] (5)
24	ip	324	105.9±12.0 (6)	0.48±.20 (4)	0.32±.06[b] (4)

Values are the means ± SEM for 4-10 independent determinations.

[a] $p<.05$
[b] $p<.01$

tion reduced ALA synthetase activity to 12, 51 and 71% of control values, respectively. The in vivo potency of heme and bilirubin as compared with Fe/dextran suggest that either or both could be the physiological regulator of ALA synthetase (Model I and IV of Figure I).

Experiments with newborn rat pups given EDTA and AIA resulted in no increases in ALA synthetase activity; the previously reported

TABLE III

Induction of Hepatic Microsomal Heme Oxygenase by Heme and Cobalt/Dextran in Adult Rats

Treatment	Dose (μmole/kg)	Time (hr)	Microsomal Heme Oxygenase Activity (nmole/mg protein/10 min)
Saline	-	4	0.60±0.04 (3)
Co/dextran	324.0	4	1.86±0.59 (4)
Heme	32.4	4	2.82±0.29[b] (4)
Heme plus cycloheximide	32.4 7.1	4	0.70±0.10 (4)
Saline	-	24	0.43±0.12 (3)
Co/dextran	324.0	24	3.56±0.85[a] (3)
Heme	32.4	24	1.48±0.73 (3)

Values are the means ± SEM for 3-4 independent enzyme assays.

[a] $p<.05$
[b] $p<.01$

premature induction of ALA synthetase by these agents (3) was not observed in this study. Two studies have found iron administration to increase rat ALA synthetase activity (4,11), the opposite effect expected of a putative repressor. Neither the experiments with adult rats nor the neonatal experiment involving EDTA and AIA treatment are compatible with iron being a physiological repressor of ALA synthetase (Model III of Figure I).

Previous dose response studies from this laboratory showed that iron is much less potent than cobalt; and cobalt is about 1/25 as potent as heme in inducing heme oxygenase activity (4). As iron/dextran is a heme oxygenase inducer of low potency, it is unlikely heme levels and subsequently ALA synthetase activity are controlled via heme oxygenase induction (Model II). Furthermore, heme can repress ALA synthetase activity at 1/10 the dose required to induce heme oxygenase activity (4). It is recognized that the pharmacokinetics of exogenously administered metals such as Fe or Co is greatly different from that of heme, an organometal.

Either Model I (the classical model) or Model IV are possible. More experimental work is necessary to evaluate the idea that bilirubin could modulate ALA synthetase activity in vivo. As heme is rapidly metabolized to bilirubin, much of the evidence suggesting heme as the modulator could be interpreted as consistent with bilirubin as the physiological modulator of ALA synthetase. Alternatively, regulation of ALA synthetase activity might involve more than one physiological modulator.

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DISCLAIMER

This report has been reviewed by the Health Effects Research Laboratory, U.S. Environmental Protection Agency, and approved for publication. Mention of trade names or commercial products does not constitute endorsement or recommendation for use.

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